Genetic characterization of Spondweni and Zika viruses and susceptibility of geographically distinct strains of *Aedes aegypti*, *Aedes albopictus*, and *Culex quinquefasciatus* (Diptera: Culicidae) to Spondweni virus

Andrew D. Haddow[1,2]*, Farooq Nasar[1,2], Hilda Guzman[1], Alongkot Ponlawat[3], Richard G. Jarman[4], Robert B. Tesh[1], and Scott C. Weaver[1,5]

[1]The University of Texas Medical Branch
Institute for Human Infections and Immunity, Department of Pathology and Center for Biodefense and Emerging Infectious Diseases
Galveston, Texas 77555-0609

[2]United States Army Medical Research Institute of Infectious Diseases (USAMRIID)
Entomology Department
Fort Detrick, MD 21702-5011

[3]Armed Forces Research Institute of Medical Sciences (AFRIMS)
Department of Entomology
Bangkok, Thailand

[4]Armed Forces Research Institute of Medical Sciences (AFRIMS)
Department of Virology
Bangkok, Thailand

[5]The University of Texas Medical Branch
Institute for Human Infections and Immunity, Department of Microbiology & Immunology
Galveston, Texas 77555-0609

*Corresponding author
Email: andrew.d.haddow.ctr@mail.mil

Abstract

Background: Zika virus (ZIKV) has extended its known geographic distribution to the Western Hemisphere and is now responsible for severe clinical complications in a sub-set of patients. While substantial genetic and vector susceptibility data exist for ZIKV, less is known for its sister flavivirus, Spondweni virus (SPONV). Both ZIKV and SPONV have been known to circulate in Africa since the mid-1900s, but neither has been genetically characterized by gene and compared in parallel. Furthermore, the susceptibility of cosmopolitan mosquito species incriminated or suspected in the transmission of ZIKV to SPONV was unknown.

Methodology/Principal Findings: In this study, two geographically distinct strains of SPONV were genetically characterized and compared to nine genetically and geographically distinct ZIKV strains. Additionally, the susceptibility of both SPONV strains was determined in three mosquito species. The open reading frame (ORF) of the SPONV 1952 Nigerian Chuku strain, exhibited a nucleotide and amino acid identity of 97.8% and 99.2%, respectively, when compared to the SPONV 1954 prototype South African AR 94 strain. The ORF of the SPONV Chuku strain exhibited a nucleotide and amino acid identity that ranged from 68.3%-69.0% and 74.6%-75.0%, respectively, when compared to nine geographically and genetically distinct strains of ZIKV. The ORF of the nine African and Asian lineage ZIKV strains exhibited limited nucleotide divergence.

Aedes aegypti, *Ae. albopictus*, and *Culex quinquefasciatus* susceptibility and dissemination was low or non-existent following artificial blood feeding of moderate doses of both SPONV strains.

Conclusions/Significance: SPONV and ZIKV nucleotide and amino acid divergence coupled with differences in geographic distribution, ecology and vector species support previous reports that these viruses are separate species. Furthermore, the low degree of SPONV dissemination in *Ae. albopictus*, *Ae. aegypti*, and *Cx. quinquefasciatus* following exposure to two geographically and genetically distinct virus strains suggest a low potential for these species to serve as vectors.

Keywords

Spondweni virus, Zika virus, mosquito, arbovirus, sylvatic, febrile, host range

Author Summary

Spondweni virus (SPONV) is a mosquito-transmitted flavivirus reported in Africa. Human infection with SPONV may result in a febrile illness similar to symptomatic Zika virus (ZIKV) infection, as well as many other tropical infections. Previously, little was known about the genetic relationships between SPONV and ZIKV. Additionally, the ability of SPONV to infect cosmopolitan mosquito species associated or incriminated in ZIKV transmission was unknown. Both SPONV strains exhibited a high degree of nucleotide and amino acid identity to each other, but considerable nucleotide and amino acid divergence to ZIKV. The open reading frame (ORF) of the nine African and Asian lineage ZIKV strains originally isolated in West Africa, Central Africa, East Africa, Southeast Asia, the Pacific Islands, and the Western Hemisphere all exhibited limited nucleotide divergence. Both strains of SPONV exhibited a low degree of infection and dissemination in *Aedes albopictus*, *Ae. aegypti*, and *Culex quinquefasciatus* mosquitoes suggesting that these species have a low potential to serve as vectors. These results coupled with differences in geographic distribution, ecology and vector species indicate that SPONV and ZIKV are similar but separate species.

Introduction

The Spondweni serogroup, genus *Flavivirus* (*Flavivirdae*), includes two species – Zika virus (ZIKV) and Spondweni virus (SPONV) [1]. Both ZIKV and SPONV are associated with human illness [2]. SPONV can cause a self-limiting febrile illness characterized by headache, myalgia, nausea, and arthralgia [3-6], signs and symptoms similar to most reported symptomatic ZIKV infections [7-12], making diagnosis challenging in those regions of Africa with virus co-circulation. Although SPONV is not typically associated with serious disease, a sub-set of patients report: conjunctivitis, macropapular and pruritic rash suggestive of vascular leakage; while reports of headache, vertigo, photophobia, disorientation, bilateral transient ocular paresis, and menigismus point to neurological involvement [3-6,13]. The close genetic relationship and the similarity in those signs and symptoms observed in typical ZIKV infections suggest the possibility of a low incidence of more severe neurologic disease.

In 1952, the Chuku strain of SPONV was isolated from the blood of a febrile patient in Nigeria [5]. This strain was initially misclassified as ZIKV [14], leading to the 1955 South African AR 94 *Mansonia uniformis* mosquito isolate being classified as the prototype SPONV strain [15]. Since its initial isolation, SPONV activity has been reported throughout sub-Saharan Africa (Table 1). In nature the virus is likely maintained in a zoonotic primate/mosquito cycle like that of ZIKV [14], and has been isolated from several mosquito genera (Table 1).

Like other flaviviruses SPONV has a positive-sense single stranded RNA genome of approximately 11 kilobases in length [16]. The genome contains 5′ and 3′ untranslated regions flanking a single open reading frame (ORF) that encodes a polyprotein that is cleaved into three structural proteins: the capsid (C), premembrane/membrane (prM), and envelope (E), and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, 2K, NS4B, and NS5) [16].

We further characterize SPONV strains and investigate their potential for urban emergence as seen with ZIKV as well as with other flaviviruses including yellow fever and dengue viruses [17,18]. We determined the genetic relationship between the prototype South African AR 94 and the Nigerian Chuku sequences of SPONV and compared those sequence data to nine geographically and genetically distinct strains of ZIKV. We also determined the susceptibility of both SPONV strains to three mosquito species that have been incriminated or suspected in the transmission of ZIKV: *Aedes aegypti* [19-21], *Ae. albopictus* [19,22], and *Culex quinquefasciatus* (C. F. Junqueira Ayres pers. comm.).

Methods

Virus strains and virus propagation

Virus strains were obtained from the World Reference Center of Emerging Viruses and Arboviruses Collection at the University of Texas Medical Branch in Galveston, Texas.

Both the South African AR 94 and Nigerian Chuku strains prior passage histories were unknown and therefore could exhibit passage-associated mutations. For this study, each virus was passaged once in *Ae. albopictus* cells (C6/36; ATCC #CCL-1660) for sequencing, and subsequently passaged once in African green monkey kidney cells (Vero; ATCC #CCL-81) for vector susceptibility experiments (virus stocks frozen at -80°C).

RNA preparation, genomic amplification, and sequencing

Viral RNA was extracted from cell culture supernatant using the QIAamp Viral RNA Kit (Qiagen, Valencia, CA, USA). Overlapping primer pairs were used to amplify the entire open reading frame (ORF) using the Titan OneStep RT-PCR kit (Roche, Mannheim, Germany) and purified amplicons were directly sequenced using the Applied Biosystems BigDye Terminator version 3.1 Cycle Sequencing Kit (Foster City, CA, USA) and the Applied Biosystems 3100 Genetic Analyzer (Foster City, CA, USA). Nucleotide sequences derived from both SPONV strains were assembled in Vector NTI Suite (Invitrogen, Carlsbad, CA, USA), aligned in SeaView [23] using MUSCLE [24], and edited in MacVector (Apex, NC, USA). These consensus sequences were deposited in GenBank, SPONV Chuku accession no. KX227369 and SPONV AR 94 accession no. KX227370.

Genetic analyses

ZIKV strains currently fall into either the African or Asian lineages [11,25]; as such nine geographically and genetically distinct sequences (i.e. strains) were used as representative

137 members of these lineages for nucleotide and amino acid comparisons with both SPONV
138 strains. The selected strains were isolated in West Africa (n = 1), Central Africa (n = 1),
139 East Africa (n = 1), Southeast Asia (n= 2), the Pacific Islands (n = 2), and the Western
140 Hemisphere (n = 2). These strains include the prototype strain MR-766 (Uganda 1947)
141 GenBank accession no. AY632535 [16]; ArB 13565 (Central African Republic 1976)
142 GenBank accession no. KF268948.1 [26]; ArD 41519 (Senegal 1984) GenBank
143 accession no. HQ234501.1 [11]; P6-740 (Malaysia 1968) GenBank accession no.
144 HQ234499 [11]; CPC-0740 (Philippines 2010) GenBank accession no. KM851038.1; EC
145 Yap (Yap Island 2007) GenBank accession no. EU545988.1 [27]; H/FP/2013 (French
146 Polynesia 2013) GenBank accession no. KJ776791.1 [28]; Z1106033 (Suriname 2015)
147 GenBank accession no. KU312312.1 [29]; and PRVABC59 (Puerto Rico 2015) GenBank
148 accession no. KU501215.1 [30]. The MR-766 sequence used in these analyses exhibited
149 a deletion the potential glycosylation site that has been noted previously [11,16].
150
151 Mosquito rearing, maintenance, and artificial infectious blood feeds
152 Three geographically distinct strains of both *Ae. albopictus* and *Ae. aegypti*, and one
153 strain of *Cx. quinquefasciatus* were used to determine susceptibility (Table 3).
154 Mosquitoes were reared and maintained during experiments using a 12:12 hour light/dark
155 photoperiod in approximately 80% relative humidity, and adult mosquitoes were
156 provided a 10% sucrose solution via a cotton ball. Four- to seven-day-old female
157 mosquitoes were sugar starved for 24 hours prior to infectious blood meal feeding, with
158 *Ae. albopictus* and *Cx. quinquefasciatus* having access to deionized water up to 12 hours
159 prior to feeding to reduce physiological stress.

Mosquito infections were performed in an Arthropod Containment Level-3 (ACL3) laboratory following the guidelines set forth under the Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition Appendix E (Arthropod Containment Guidelines). Groups of 100 mosquitoes were allowed to feed from artificial membrane feeders (Discovery Workshops, Lancashire, UK) covered by rat skins and containing a suspension of one part defibrinated sheep blood (Colorado Serum Company, Denver, CO, USA) and virus. Blood meal titers were 5.1 (Chuku) and 5.3 (AR 94) $\log_{10}$PFU/mL. Post feeding, mosquitoes were sorted on ice and fully engorged individuals meeting the criteria for stages 4 to 5 were retained [31].

Mosquito processing and virus assay

On day 14 post-feeding, mosquitoes were chilled for immobilization, then dissected, pooled and homogenized (legs/wings and body separately) in a tubes containing a steel BB and 500µl of media [Dulbecco's Modified Eagle Medium supplemented with 20% (vol/vol) fetal bovine serum, 100 U/ml of penicillin, 100 µg/ml of streptomycin, and 0.5 mg/ml amphotericin B (Sigma Aldrich, St. Louis, MO, USA)], and frozen at -80°C. Pools were assayed on C6/36 cells for the presence of SPONV antigen by an indirect fluorescent antibody (IFA) test using hyperimmune mouse ascitic fluid (HMAF) directed against the SPONV Chuku strain and a commercial fluorescein isothiocyanate-conjugated goat antimouse immunoglobulin G (Sigma Aldrich, St. Louis, MO, USA) [32,33].

Results

Genetic analysis

The complete sequence of both SPONV strains was translated and aligned with selected ZIKV strains. The ORF of SPONV AR 94 and Chuku strains displayed >98% nucleotide and amino acid identity to each other, whereas they displayed ~68% and ~75% percent nucleotide and amino acid identity to ZIKV. Next we compared nucleotide and amino acid identity in the individual genes of SPONV and ZIKV. The lengths of individual genes were determined by utilizing putative cleavage sites of ZIKV genes. The individual SPONV gene sizes were similar to ZIKV genes: Capsid, prM, NS1, NS4A, and NS5 were identical, whereas the E (505 vs. 504 amino acid), NS2A (226 vs. 217 amino acid), NS2B (130 vs. 122 amino acid), NS3 (619 vs. 617 amino acid) and NS4B (255 vs. 251 amino acid) were larger than ZIKV. The individual structural gene comparison of SPONV and ZIKV showed nucleotide and amino acid identity ranging from 61% to 68% and 64% to 72%, respectively, with the E gene displaying greater sequence identity (68% nucleotide and 72% amino acid). The nonstructural gene comparison displayed nucleotide and amino acid identity ranging from 59% to 73% and 58% to 82%, respectively. The NS4B and NS3 genes displayed the greater identity, 70% to 72% nucleotide and 81-82% amino acid. The NS2A gene was the most divergent gene with 59% to 60% nucleotide and 58% to 59% amino acid identity between SPONV and ZIKV.

Mosquito infection and dissemination

Exposure to the SPONV Chuku strain by artificial blood meal did not result in any infection or dissemination in any of the three mosquito species (Table 2). Exposure to the SPONV AR 94 strain by artificial blood meal resulted infection in 8.3% of *Ae. aegypti* (Galveston) and 12.5% *Ae. aegypti* (Thailand), while only *Ae. aegypti* (Galveston) developed disseminated infection (8.3%).

Discussion

Prior to this study, one SPONV strain had been sequenced, but its geographic origin and passage history was not reported [34]. Our analyses demonstrated that both SPONV strains sequenced in this study (Chuku and AR 94) are genetically similar, but exhibit a high degree of nucleotide and amino acid divergence when compared to ZIKV strains from West Africa, East Africa, Southeast Asia, the Pacific Islands, and the Western Hemisphere (Fig. 1). The similarity between the two SPONV strains isolated in different geographic regions approximately 2.5 years apart may indicate the possibility of continuous enzootic transmission and maintenance between Nigeria and South Africa, although interpretation is limited due to the lack of spatial and temporally spaced sequences (i.e. multiple isolates). ZIKV strains within each lineage, African and Asian, also exhibited a low degree of nucleotide divergence when compared to one another, as seen in previous work [11]. With the exception of the MR-766 ZIKV strain, neither SPONV strain nor any of the other eight ZIKV strains used in this study exhibited a

228 deletion in the potential N-linked glycosylation site as reported in some ZIKV strains that
229 had prior passage histories in mouse brains [11,16,26,35].
230
231 The susceptibility and dissemination to moderate doses of both SPONV strains in all
232 three species was low or non-existent (Table 3). The Chuku strain did not cause any
233 infection/dissemination in any of the species, while the AR 94 strain was only observed
234 to cause disseminated infection in *Ae. albopictus* Galveston (8.3%). Work by Bearcroft
235 also failed to show transmission of the Chuku strain by *Ae. aegypti* [3]. Unlike SPONV,
236 *Ae. aegypti* and *Ae. albopictus* have been incriminated as vectors of ZIKV [19-22] and
237 recently *Cx. quinequefaciatus* has been discussed as a potential vector in Brazil (C. F.
238 Junqueira Ayres pers. comm.). Early work demonstrated that *Ae. aegypti* was a
239 competent vector of ZIKV following feeding on an artificial blood meal containing the
240 MR-766 prototype strain, with three mosquitoes transmitting ZIKV to a single rhesus
241 monkey 72 days post-exposure [21]. Since that time, several studies have shown that
242 various geographically distinct strains of *Ae. aegypti* or *Ae. albopictus* mosquitoes
243 exposed to ZIKV strains from either the African and Asian lineages exhibit a wide range
244 of susceptibility and/or vector competence in these two mosquito species [19,20,22,36].
245 Caution should be exercised regarding the over interpretation of the results of vector
246 susceptibility/competence studies, as variation in vector competence between
247 geographically distinct mosquito populations has been reported in other arboviruses [37].
248 Also, many of these studies used very high passage ZIKV strains.
249

Unlike its sister ZIKV, which has a broad geographic distribution, SPONV isolations and seroprevalence have thus far been confined to Africa (Table 1) [11,25,38]. While it is possible that the difference in the geographic distribution between ZIKV and SPONV is a result of prior infection with ZIKV or SPONV resulting in a refractory status among amplification hosts, another explanation is there are differences in the vector species between these two viruses. Intensive mosquito collections and subsequent virus isolation attempts over a number of years by laboratories in sub-Saharan Africa yielded isolations of SPONV from eight species of mosquitoes in the genera *Aedes*, *Culex*, *Eretmapodities*, and *Mansonia* (Table 1), while ZIKV has been isolated in 20 species in the genera *Aedes*, *Anopheles*, *Eretmapodities*, and *Mansonia* [11]. Although many of these species are found in the same regions where both SPONV and ZIKV have been isolated, both viruses have only been isolated in 2 species, *Ae. fowleri* and *Ma. uniformis*. Further studies are needed to determine the potential for sylvatic mosquito species to transmit both ZIKV and SPONV.

Previous to the Ninth Report of the International Committee on the Taxonomy of Viruses (ICTV) [39], SPONV was considered a species of the Genus *Flavivirus*: Family *Flaviviridae*, and both SPONV and ZIKV were considered members of the Spondweni Serogroup [2]. According to the current report, SPONV has now been categorized as a member of the genus *Flavivirus* that has not been approved as a species. SPONV clearly exhibits a greater nucleotide (~ 32%) and amino acid (~25%) divergence from its sister virus – ZIKV as has been previously reported (Fig. 1) [26]. This is particularly evident when comparing individual proteins rather than the entire ORF (Figs 2, 3, 4).

Comprehensive historic work using neutralization, hemaggutination-inhibition, complement fixation, and antibody absorption tests also differentiate SPONV and ZIKV as distinct viruses based on limited cross-reactivity [2,14,40,41]. Furthermore, both viruses exhibit differences in vector associations, ecology, and geographic distribution. These data suggest that although both SPONV and ZIKV are related, they are separate species.

In conclusion, this study determined the genetic relationship between two sequences of SPONV, as well as to nine representative African and Asian lineage ZIKV strains. The SPONV Chuku and AR 94 strains exhibited poor infection and dissemination in *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefaciatus* mosquitoes, indicating a low potential for these species to serve as vectors and probably limited emergence potential into urban cycles characteristic of ZIKV, yellow fever virus, and dengue viruses. Nucleotide and amino acid divergence coupled with differences in geographic distribution, ecology and vector species support previous reports that SPONV and ZIKV are separate species.

Acknowledgements

The authors wish to thank Jason Richardson for his assistance in coordinating work between UTMB and AFRIMS.

Disclosure Statement

The views expressed in this article are those of the authors and do not reflect the official policy or position of the U.S. Department of Defense, the Department of the Army, Centers for Disease Control and Prevention, or the Kingdom of Thailand.

Contributor information

Andrew D. Haddow, Email: adhaddow@gmail.com or andrew.d.haddow.ctr@mail.mil

Farooq Nasar, Email: farooq.nasar.ctr@mail.mil

Hilda Guzman, Email: hguzman@utmb.edu

Alongkot Ponlawat, Email: AlongkotP.fsn@afrims.org

Richard H. Jarman, Email: richard.g.jarman.mil@mail.mil

Robert B. Tesh, Email: rtesh@utmb.edu

Scott C. Weaver, Email: sweaver@utmb.edu

References

1. Casels J (1957) The arthropod-borne group of animal viruses. Trans NY Acad Sci 19: 219-235.
2. Theiler M, Downs WG (1973) The arthropod-borne viruses of vertebrates. New Haven and London: Yale University Press.
3. Bearcroft WG (1956) Zika virus infection experimentally induced in a human volunteer. Trans R Soc Trop Med Hyg 50: 442-448.
4. Draper CC (1965) Infection with the Chuku Strain of Spondweni virus. West Afr Med J 14: 16-19.
5. Macnamara FN (1954) Zika virus: a report on three cases of human infection during an epidemic of jaundice in Nigeria. Trans R Soc Trop Med Hyg 48: 139-145.
6. McIntosh BM, Kokernot RH, Paterson HE, De Meillon B (1961) Isolation of Spondweni virus from four species of Culicine mosquitoes and a report of two laboratory infections with the virus. S Afr Med J 35: 647-650.
7. Foy BD, Kobylinski KC, Chilson Foy JL, Blitvich BJ, Travassos da Rosa A, et al. (2011) Probable non-vector-borne transmission of Zika virus, Colorado, USA. Emerg Infect Dis 17: 880-882.
8. Simpson DI (1964) Zika virus infection in man. Trans R Soc Trop Med Hyg 58: 335-338.
9. Filipe AR, Martins CM, Rocha H (1973) Laboratory infection with Zika virus after vaccination against yellow fever. Arch Gesamte Virusforsch 43: 315-319.
10. Olson JG, Ksiazek TG, Suhandiman, Triwibowo (1981) Zika virus, a cause of fever in Central Java, Indonesia. Trans R Soc Trop Med Hyg 75: 389-393.
11. Haddow AD, Schuh AJ, Yasuda CY, Kasper MR, Heang V, et al. (2012) Genetic characterization of Zika virus strains: geographic expansion of the Asian lineage. PLoS Negl Trop Dis 6: e1477.
12. Heang V, Yasuda CY, Sovann L, Haddow AD, Travassos da Rosa AP, et al. (2012) Zika virus infection, Cambodia, 2010. Emerg Infect Dis 18: 349-351.
13. Wolfe MS, Calisher CH, McGuire K (1982) Spondweni virus infection in a foreign resident of Upper Volta. Lancet 2: 1306-1308.
14. Haddow AJ, Williams MC, Woodall JP, Simpson DI, Goma LK (1964) Twelve Isolations of Zika Virus from *Aedes* (Stegomyia) *africanus* (Theobald) Taken in and above a Uganda Forest. Bull World Health Organ 31: 57-69.
15. Kokernot RH, Smithburn KC, Muspratt J, Hodgson B (1957) Studies on arthropod-borne viruses of Tongaland. VIII. Spondweni virus, an agent previously unknown, isolated from *Taeniorhynchus* (Mansonioides) *uniformis*. S Afr J Med Sci 22: 103-112.
16. Kuno G, Chang GJ (2007) Full-length sequencing and genomic characterization of Bagaza, Kedougou, and Zika viruses. Arch Virol 152: 687-696.
17. Vasilakis N, Cardosa J, Hanley KA, Holmes EC, Weaver SC (2011) Fever from the forest: prospects for the continued emergence of sylvatic dengue virus and its impact on public health. Nat Rev Microbiol 9: 532-541.
18. Hanley KA, Monath TP, Weaver SC, Rossi SL, Richman RL, et al. (2013) Fever versus fever: the role of host and vector susceptibility and interspecific

competition in shaping the current and future distributions of the sylvatic cycles of dengue virus and yellow fever virus. Infect Genet Evol 19: 292-311.
19. Chouin-Carneiro T, Vega-Rua A, Vazeille M, Yebakima A, Girod R, et al. (2016) Differential susceptibilities of *Aedes aegypti* and *Aedes albopictus* from the Americas to Zika Virus. PLoS Negl Trop Dis 10: e0004543.
20. Li MI, Wong PS, Ng LC, Tan CH (2012) Oral susceptibility of Singapore Aedes (Stegomyia) aegypti (Linnaeus) to Zika virus. PLoS Negl Trop Dis 6: e1792.
21. Boorman JP, Porterfield JS (1956) A simple technique for infection of mosquitoes with viruses; transmission of Zika virus. Trans R Soc Trop Med Hyg 50: 238-242.
22. Wong PS, Li MZ, Chong CS, Ng LC, Tan CH (2013) *Aedes* (Stegomyia) *albopictus* (Skuse): a potential vector of Zika virus in Singapore. PLoS Negl Trop Dis 7: e2348.
23. Galtier N, Gouy M, Gautier C (1996) SEAVIEW and PHYLO_WIN: two graphic tools for sequence alignment and molecular phylogeny. Comput Appl Biosci 12: 543-548.
24. Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32: 1792-1797.
25. Plourde AR, Bloch EV (2016) A literature review of Zika virus. Emerg Infect Dis 22.
26. Berthet N, Nakoune E, Kamgang B, Selekon B, Descorps-Declere S, et al. (2014) Molecular characterization of three Zika flaviviruses obtained from sylvatic mosquitoes in the Central African Republic. Vector Borne Zoonotic Dis 14: 862-865.
27. Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, et al. (2008) Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. Emerg Infect Dis 14: 1232-1239.
28. Baronti C, Piorkowski G, Charrel RN, Boubis L, Leparc-Goffart I, et al. (2014) Complete coding sequence of zika virus from a French polynesia outbreak in 2013. Genome Announc 2.
29. Enfissi A, Codrington J, Roosblad J, Kazanji M, Rousset D (2016) Zika virus genome from the Americas. Lancet 387: 227-228.
30. Lanciotti RS, Lambert AJ, Holodniy M, Saavedra S, del Carmen Castillo Signor L (2016) Phylogeny of Zika virus in Western Hemisphere, 2015. Emerg Infect Dis In Press.
31. Pilitt DR, Jones JC (1972) A qualitative method for estimating the degree of engorgement of *Aedes aegypti* adults. J Med Entomol 9: 334-337.
32. Tesh RB (1979) A method for the isolation of dengue viruses, using mosquito cell cultures. Am J Trop Med Hyg 28: 1053-1059.
33. Tesh RB, Guzman H, da Rosa AP, Vasconcelos PF, Dias LB, et al. (2001) Experimental yellow fever virus infection in the Golden Hamster (*Mesocricetus auratus*). I. Virologic, biochemical, and immunologic studies. J Infect Dis 183: 1431-1436.
34. Grard G, Moureau G, Charrel RN, Holmes EC, Gould EA, et al. (2010) Genomics and evolution of Aedes-borne flaviviruses. J Gen Virol 91: 87-94.
35. Faye O, Freire CC, Iamarino A, Faye O, de Oliveira JV, et al. (2014) Molecular evolution of Zika virus during its emergence in the 20(th) century. PLoS Negl Trop Dis 8: e2636.

36. Diagne CT, Diallo D, Faye O, Ba Y, Faye O, et al. (2015) Potential of selected Senegalese Aedes spp. mosquitoes (Diptera: Culicidae) to transmit Zika virus. BMC Infect Dis 15: 492.
37. Tesh RB, Gubler DJ, Rosen L (1976) Variation among goegraphic strains of *Aedes albopictus* in susceptibility to infection with chikungunya virus. Am J Trop Med Hyg 25: 326-335.
38. Petersen LR, Jamieson DJ, Powers AM, Honein MA (2016) Zika Virus. N Engl J Med.
39. (2012) Virus taxonomy: classification and nomenclature of viruses: Ninth Report of the International Committee on Taxonomy of Viruses. San Diego, CA, USA.
40. Casals J (1961) Procedures for identification of arthropod-borne viruses. Bull World Health Organ 24: 723-734.
41. Clarke DH, Casals J (1958) Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. Am J Trop Med Hyg 7: 561-573.
42. Kokernot RH, Casaca VM, Weinbren MP, McIntosh BM (1965) Survey for antibodies against arthropod-borne viruses in the sera of indigenous residents of Angola. Trans R Soc Trop Med Hyg 59: 563-570.
43. Kokernot RH, Szlamp EL, Levitt J, McIntosh BM (1965) Survey for antibodies against arthropod-borne viruses in the sera of indigenous residents of the Caprivi Strip and Bechuanaland Protectorate. Trans R Soc Trop Med Hyg 59: 553-562.
44. Brottes H, Rickenbach A, Bres P, Salaun JJ, Ferrara L (1966) [Arboviruses in the Cameroon. Isolation from mosquitoes]. Bull World Health Organ 35: 811-825.
45. Ardoin P, Rodhain F, Hannoun C (1976) Epidemiologic study of arboviruses in the Arba-Minch district of Ethiopia. Trop Geogr Med 28: 309-315.
46. Kokernot RH, Smithburn KC, Gandara AF, McIntosh BM, Heymann CS (1960) [Neutralization tests with sera from individuals residing in Mozambique against specific viruses isolated in Africa, transmitted by arthropods]. An Inst Med Trop (Lisb) 17: 201-230.
47. Worth CB, Paterson HE, De Meillon B (1961) The incidence of arthropod-borne viruses in a population of culicine mosquitoes in Tongaland, Union of South Africa (January, 1956, through April, 1960). Am J Trop Med Hyg 10: 583-592.
48. McIntosh BM, Jupp PG, De Sousa J (1972) Further isolations of the arboviruses from mosquitoes collected in Tongaland, South Africa, 1960-1968. J Med Entomol 9: 155-159.

Table 1. Reported geographic distribution of Spondweni virus*.

Country	Seroprevelance† (Humans)	Virus isolation (Human)	Virus isolation (Mosquito)	Reference(s)
Angola	X			[42]
Botswana	X			[43]
Burkina Faso	X			[13]
Cameroon	X	X	*Eretmapodites spp.*	[13,44]
Ethiopia	X			[45]
Gabon	X			[13]
Mozambique	X		*Aedes fryeri/fowleri*	[46]
Namibia	X			[43]
Nigeria	X	X		[5]
South Africa	X		*Ae. circumluteolus, Ae.cumminsi, Culex neavi, Cx. univittatus, Er. silvestris, Mansonia africana, Ma. uniformis*	[6,15,47,48]

*Does not include laboratory acquired infections.

† Seroprevalence was determined by one or more of the following methods: Haemagglution inhibition, neutralization, and/or complement-fixation.

Of note, it is possible due to antigenic cross-reactivity among flaviviruses that seropositive individuals may have been previously exposed to one or more flaviviruses and not to Spondweni virus.

Table 2. Mosquito susceptibility to Spondweni virus.

A. Susceptibility of selected mosquito species to Spondweni Chuku strain, dose 5.1 log_{10}PFU/mL.

Mosquito (origin)	No.	Infection, no. (%)	Dissemination, no. (%)
Aedes aegypti (Galveston, USA)	19	0 (0.0)	0 (0.0)
Aedes aegypti (Iquitos, Peru)	20	0 (0.0)	0 (0.0)
Aedes aegypti (Thailand)	4	0 (0.0)	0 (0.0)
Aedes albopicutus (Galveston, USA)	24	0 (0.0)	0 (0.0)
Aedes albopicutus (Thailand)	12	0 (0.0)	0 (0.0)
Aedes albopicutus (Venezuela)	3	0 (0.0)	0 (0.0)
Culex quinquefaciatus (Galveston, USA)	24	0 (0.0)	0 (0.0)

B. Susceptibility of selected mosquito species to Spondweni AR 94 strain, dose 5.3 log_{10}PFU/mL.

Mosquito	No.	Infection, no. (%)	Dissemination, no. (%)
Aedes aegypti (Galveston, USA)	24	0 (0.0)	0 (0.0)
Aedes aegypti (Iquitos, Peru)	24	0 (0.0)	0 (0.0)
Aedes aegypti (Thailand)	24	1 (4.2)	0 (0.0)
Aedes albopicutus (Galveston, USA)	24	2 (8.3)	2 (8.3)
Aedes albopicutus (Thailand)	24	3 (12.5)	0 (0.0)
Aedes albopicutus (Venezuela)	24	0 (0.0)	0 (0.0)
Culex quinquefaciatus (Galveston, USA)	24	0 (0.0)	0 (0.0)

Figure 1. Genome structure and pairwise comparison of the open reading frame (ORF) of Spondweni (SPONV) and Zika (ZIKV) viruses.*

A) SPONV genome organization: capsid (C), premembrane/membrane (prM), envelope (E), NS1, NS2A, NS2B, NS3, NS4A, 2K (not shown), NS4B, and NS5. Numbers indicate animo acids in each protein.

B) Pairwise comparison of the ORF of SPONV and ZIKV strains. SPONV AR 94; SPONV Chuku; ZIKV MR-766; ZIKV ArB 13565; ZIKV ArD 41519; ZIKV P6-740; ZIKV CPC-0740; ZIKV EC Yap; ZIKV H/PF/2013; ZIKV Z1106033; ZIKV PRVABC59. *Boldface type (upper diagonal) = Percent amino acid identity; Lightface type (lower diagonal) = Percent nucleotide identity.

Figure 2. Pairwise comparison of the structural proteins of Spondweni (SPONV) and Zika (ZIKV) viruses.*

Capsid (C), premembrane/membrane (prM), and envelope (E). SPONV AR 94; SPONV Chuku; ZIKV MR-766; ZIKV ArB 13565; ZIKV ArD 41519; ZIKV P6-740; ZIKV CPC-0740; ZIKV EC Yap; ZIKV H/PF/2013; ZIKV Z1106033; ZIKV PRVABC59. *Boldface type (upper diagonal) = Percent amino acid identity; Lightface type (lower diagonal) = Percent nucleotide identity.

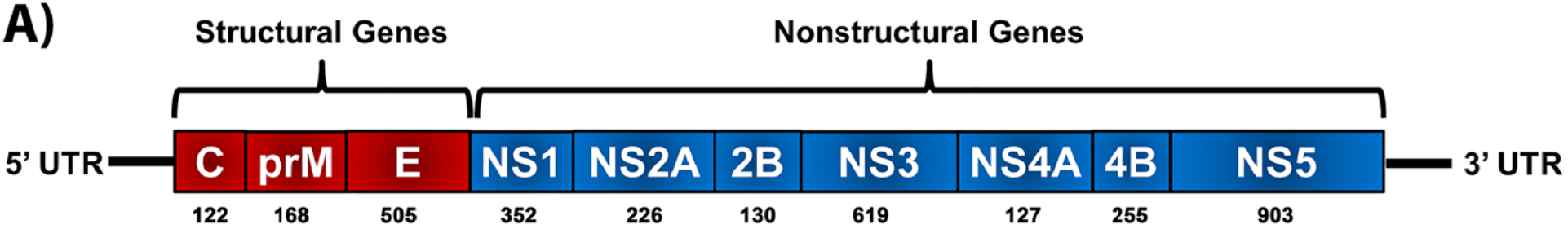
Figure 3. Pairwise comparison of the non-structural proteins NS2b, NS3, and NS5 of Spondweni (SPONV) and Zika (ZIKV) viruses.*

SPONV AR 94; SPONV Chuku; ZIKV MR-766; ZIKV ArB 13565; ZIKV ArD 41519; ZIKV P6-740; ZIKV CPC-0740; ZIKV EC Yap; ZIKV H/PF/2013; ZIKV Z1106033; ZIKV PRVABC59. *Boldface type (upper diagonal) = Percent amino acid identity; Lightface type (lower diagonal) = Percent nucleotide identity.

Figure 4. Pairwise comparison of the non-structural proteins NS1, NS2a, NS4a, and NS4b of Spondweni (SPONV) and Zika (ZIKV) viruses.*

SPONV AR 94; SPONV Chuku; ZIKV MR-766; ZIKV ArB 13565; ZIKV ArD 41519; ZIKV P6-740; ZIKV CPC-0740; ZIKV EC Yap; ZIKV H/PF/2013; ZIKV Z1106033; ZIKV PRVABC59. *Boldface type (upper diagonal) = Percent amino acid identity; Lightface type (lower diagonal) = Percent nucleotide identity.

A)



B)

ORF	*AR 94*	*Chuku*	MR-766	ArB 13565	ArD 41519	P6-740	CPC-0740	EC YAP	H/PF/2013	Z1106033	PRVABC59
AR 94	-	99.2	74.6	75.0	74.9	74.8	74.9	74.6	74.8	74.8	74.7
Chuku	97.8	-	74.6	75.0	74.9	74.8	75.0	74.7	74.9	74.8	74.8
MR-766	68.5	68.5	-	98.4	98.4	97.0	96.4	96.4	96.5	96.4	96.4
ArB 13565	68.9	68.8	94.8	-	98.9	97.6	97.2	96.8	97.2	97.1	97.1
ArD 41519	69.0	69.0	93.1	93.2	-	97.4	97.0	96.7	97.1	97.0	97.0
P6-740	68.4	68.5	89.8	90.0	90.0	-	98.9	98.6	98.9	98.9	98.9
CPC-0740	68.2	68.3	88.4	88.6	88.5	95.6	-	99.2	99.4	99.4	99.4
EC YAP	68.2	68.3	88.7	88.6	88.6	95.8	98.2	-	99.2	99.1	99.1
H/PF/2013	68.3	68.4	88.5	88.7	88.6	95.6	97.9	98.1	-	99.9	99.9
Z1106033	68.2	68.3	88.4	88.7	88.5	95.4	97.6	97.9	99.7	-	99.9
PRVABC59	68.2	68.3	88.5	88.8	88.6	95.5	97.6	97.9	99.7	99.7	-

C

	AR 94	Chuku	MR-766	ArB 13565	ArD 41519	P6-740	CPC-0740	EC YAP	H/PF/2013	Z1106033	PRVABC59
AR 94	-	97.5	64.5	66.1	66.1	66.9	66.9	65.3	66.1	66.1	65.3
Chuku	96.4	-	65.3	66.9	66.9	67.7	67.7	66.1	66.9	66.9	66.1
MR-766	63.4	64.2	-	95.1	94.3	90.2	90.2	94.3	91.1	91.1	90.2
ArB 13565	65.1	65.3	94.3	-	98.4	95.1	95.1	89.4	95.9	95.9	95.1
ArD 41519	64.5	65.3	91.3	94.0	-	95.1	95.1	90.2	95.9	95.9	95.1
P6-740	64.8	65.1	89.7	92.1	92.1	-	100.0	94.3	99.2	99.2	98.4
CPC-0740	64.8	65.6	89.2	91.3	91.3	96.7	-	94.3	99.2	99.2	98.4
EC YAP	64.2	65.1	91.9	88.6	87.8	94.6	95.7	-	93.5	93.5	92.7
H/PF/2013	65.3	65.6	88.9	91.6	90.8	97.0	98.1	95.9	-	100.0	99.2
Z1106033	65.3	65.6	88.9	91.6	90.8	97.0	98.1	95.9	100.0	-	99.2
PRVABC59	65.1	65.3	88.6	91.3	90.5	96.7	97.8	95.7	99.7	99.7	-

prM

	AR 94	Chuku	MR-766	ArB 13565	ArD 41519	P6-740	CPC-0740	EC YAP	H/PF/2013	Z1106033	PRVABC59
AR 94	-	99.4	64.3	63.7	64.3	64.3	64.9	64.3	64.3	63.7	64.3
Chuku	98.0	-	64.9	64.3	64.9	64.9	65.5	64.9	64.9	64.3	64.9
MR-766	62.7	62.3	-	98.8	100.0	95.2	94.0	94.6	94.0	93.4	94.0
ArB 13565	61.9	61.5	94.8	-	98.8	95.2	94.0	94.6	94.0	93.4	94.0
ArD 41519	62.3	63.1	91.8	91.2	-	95.2	94.0	94.6	94.0	93.4	94.0
P6-740	61.7	62.5	88.8	88.0	86.0	-	98.8	99.4	98.8	98.2	98.8
CPC-0740	61.9	63.1	87.2	87.0	85.0	95.6	-	99.4	98.8	98.2	98.8
EC YAP	61.3	62.9	88.0	87.8	85.8	96.0	98.0	-	99.4	98.8	99.4
H/PF/2013	61.3	62.5	88.0	87.4	85.8	95.6	96.4	97.2	-	99.4	100.0
Z1106033	60.9	62.1	88.0	87.4	85.8	95.6	96.4	97.2	99.6	-	99.4
PRVABC59	61.1	62.3	88.2	87.6	86.0	95.6	96.4	97.2	99.6	99.6	-

E

	AR 94	Chuku	MR-766	ArB 13565	ArD 41519	P6-740	CPC-0740	EC YAP	H/PF/2013	Z1106033	PRVABC59
AR 94	-	99.2	71.5	72.7	72.5	72.5	72.5	72.3	72.5	72.7	72.5
Chuku	97.8	-	71.5	72.9	72.5	72.1	72.3	72.1	72.3	72.5	72.3
MR-766	69.1	68.4	-	98.2	98.4	96.8	96.8	96.6	96.4	96.2	96.4
ArB 13565	69.6	69.0	94.0	-	99.6	98.0	98.0	97.8	97.6	97.4	97.6
ArD 41519	68.6	68.4	92.3	93.0	-	98.2	98.4	98.2	98.0	97.8	98.0
P6-740	67.9	67.7	89.0	90.3	89.5	-	99.4	99.2	99.0	98.8	99.0
CPC-0740	68.3	68.0	87.3	88.2	88.1	95.5	-	99.8	99.6	99.4	99.6
EC YAP	68.0	67.5	87.7	88.9	88.5	96.1	98.5	-	99.4	99.2	99.4
H/PF/2013	68.4	67.9	87.7	89.0	88.4	95.9	98.3	98.7	-	99.8	100.0
Z1106033	68.5	68.0	87.4	88.8	88.0	95.4	97.9	98.3	99.4	-	99.8
PRVABC59	68.3	67.8	87.6	88.9	88.1	95.6	98.1	98.5	99.6	99.5	-

NS2b

	AR 94	Chuku	MR-766	ArB 13565	ArD 41519	P6-740	CPC-0740	EC YAP	H/PF/2013	Z1106033	PRVABC59
AR 94	-	100.0	76.2	76.2	76.2	76.9	77.7	76.9	76.9	76.9	76.9
Chuku	99.2	-	76.2	76.2	76.2	76.9	77.7	76.9	76.9	76.9	76.9
MR-766	72.1	72.3	-	100.0	100.0	98.5	97.7	98.5	98.5	98.5	98.5
ArB 13565	72.8	73.1	95.1	-	100.0	98.5	97.7	98.5	98.5	98.5	98.5
ArD 41519	71.8	72.1	94.9	94.6	-	98.5	97.7	98.5	98.5	98.5	98.5
P6-740	71.5	72.3	91.5	93.1	92.6	-	99.2	100.0	100.0	100.0	100.0
CPC-0740	72.3	73.1	90.0	91.0	91.0	95.1	-	99.2	99.2	99.2	99.2
EC YAP	71.8	72.6	90.3	90.8	91.3	95.4	98.2	-	100.0	100.0	100.0
H/PF/2013	72.3	73.1	89.7	90.8	90.8	95.4	97.2	96.9	-	100.0	100.0
Z1106033	72.3	73.1	89.7	90.8	90.8	95.4	97.2	96.9	100.0	-	100.0
PRVABC59	72.3	73.1	89.7	90.8	90.8	95.4	97.2	96.9	100.0	100.0	-

NS3

	AR 94	Chuku	MR-766	ArB 13565	ArD 41519	P6-740	CPC-0740	EC YAP	H/PF/2013	Z1106033	PRVABC59
AR 94	-	99.7	81.1	81.4	81.3	81.4	81.3	81.1	81.3	81.3	81.3
Chuku	98.2	-	80.9	81.3	81.1	81.3	81.1	80.9	81.1	81.1	81.1
MR-766	71.9	71.9	-	99.7	99.0	98.7	98.1	98.1	98.2	98.2	98.2
ArB 13565	72.3	72.1	95.4	-	99.4	99.0	98.4	98.4	98.5	98.5	98.5
ArD 41519	72.3	72.3	93.7	93.5	-	98.4	97.7	97.7	97.9	97.9	97.9
P6-740	71.5	71.9	90.9	90.7	90.7	-	99.4	99.4	99.5	99.5	99.5
CPC-0740	70.6	70.9	88.8	88.6	89.0	95.8	-	99.0	99.2	99.2	99.2
EC YAP	70.8	71.2	88.8	88.7	89.1	96.3	98.2	-	99.2	99.2	99.2
H/PF/2013	70.8	71.1	88.9	88.9	89.3	96.3	97.5	98.1	-	100.0	100.0
Z1106033	70.8	71.0	88.8	88.8	89.0	96.1	97.2	97.9	99.7	-	100.0
PRVABC59	70.8	71.0	89.0	88.9	89.2	96.1	97.3	97.9	99.8	99.6	-

NS5

	AR 94	Chuku	MR-766	ArB 13565	ArD 41519	P6-740	CPC-0740	EC YAP	H/PF/2013	Z1106033	PRVABC59
AR 94	-	98.6	77.6	77.6	77.6	77.1	77.3	77.2	77.3	77.2	77.1
Chuku	97.7	-	77.5	77.5	77.5	77.2	77.6	77.5	77.6	77.5	77.4
MR-766	69.8	69.7	-	97.9	98.7	96.5	96.0	95.9	96.0	95.9	95.8
ArB 13565	70.0	69.8	94.9	-	98.6	96.7	96.6	96.5	96.6	96.5	96.3
ArD 41519	70.6	70.4	93.5	93.1	-	96.7	96.5	96.3	96.5	96.3	96.2
P6-740	70.0	70.0	89.7	89.2	90.0	-	98.3	98.2	98.3	98.3	98.2
CPC-0740	69.9	69.9	88.3	88.1	88.2	95.2	-	99.9	99.8	99.7	99.6
EC YAP	69.8	69.7	88.6	88.1	88.5	95.7	98.6	-	99.9	99.8	99.7
H/PF/2013	70.0	70.1	88.2	87.9	88.3	95.2	98.0	98.6	-	99.9	99.8
Z1106033	69.8	69.9	88.2	87.9	88.3	95.0	97.7	98.4	99.8	-	99.9
PRVABC59	69.9	70.0	88.3	88.0	88.4	95.1	97.7	98.4	99.7	99.9	-

NS1

	AR 94	*Chuku*	MR-766	ArB 13565	ArD 41519	P6-740	CPC-0740	EC YAP	H/PF/2013	Z1106033	PRVABC59
AR 94	-	99.4	74.1	75.0	74.4	74.1	74.4	74.1	74.4	74.4	74.4
Chuku	97.3	-	73.9	74.7	74.1	73.9	74.1	73.9	74.1	74.1	74.1
MR-766	67.9	68.8	-	98.3	98.0	97.4	97.4	96.6	97.4	97.4	97.4
ArB 13565	68.6	69.5	95.5	-	98.9	98.0	98.3	97.4	98.3	98.3	98.3
ArD 41519	69.1	69.6	93.3	93.7	-	97.7	98.0	97.2	98.0	98.0	98.0
P6-740	69.1	69.4	90.8	90.1	90.1	-	99.4	99.1	99.4	99.4	99.4
CPC-0740	68.7	68.8	90.1	89.5	89.3	95.3	-	99.1	99.4	99.4	99.4
EC YAP	68.7	68.9	90.0	89.4	89.4	95.4	98.8	-	99.1	99.1	99.1
H/PF/2013	68.8	69.0	89.8	89.3	89.4	94.6	98.2	98.7	-	100.0	100.0
Z1106033	68.7	68.9	89.8	89.3	89.4	94.5	97.9	98.4	99.7	-	100.0
PRVABC59	68.6	68.8	89.9	89.4	89.3	94.3	97.9	98.4	99.7	99.8	-

NS2a

	AR 94	*Chuku*	MR-766	ArB 13565	ArD 41519	P6-740	CPC-0740	EC YAP	H/PF/2013	Z1106033	PRVABC59
AR 94	-	99.1	59.3	58.4	58.0	57.5	57.5	57.1	57.5	57.5	57.5
Chuku	97.5	-	59.3	58.4	58.0	57.5	57.5	57.1	57.5	57.5	57.5
MR-766	59.3	59.3	-	97.3	96.9	96.0	96.0	95.6	96.0	96.0	96.0
ArB 13565	59.6	59.0	94.2	-	98.7	96.5	96.5	96.0	96.5	96.5	96.5
ArD 41519	59.7	59.4	93.1	92.9	-	96.0	96.0	95.6	96.0	96.0	96.0
P6-740	58.8	58.4	88.2	89.2	87.6	-	99.1	98.7	99.1	99.1	99.1
CPC-0740	58.3	58.1	88.1	89.2	87.2	97.1	-	99.6	100.0	100.0	100.0
EC YAP	58.8	58.7	87.3	88.1	86.3	96.3	97.8	-	99.6	99.6	99.6
H/PF/2013	58.6	58.4	87.0	88.3	86.0	96.6	97.9	97.2	-	100.0	100.0
Z1106033	58.6	58.4	87.5	88.5	86.1	96.5	97.8	97.1	99.6	-	100.0
PRVABC59	58.7	58.6	87.2	88.5	85.8	96.5	97.8	97.1	99.6	99.7	-

NS4a

	AR 94	*Chuku*	MR-766	ArB 13565	ArD 41519	P6-740	CPC-0740	EC YAP	H/PF/2013	Z1106033	PRVABC59
AR 94	-	100.0	75.6	75.6	74.8	75.6	76.4	75.6	75.6	75.6	75.6
Chuku	99.0	-	75.6	75.6	74.8	75.6	76.4	75.6	75.6	75.6	75.6
MR-766	65.1	65.1	-	99.2	99.2	99.2	97.6	97.6	99.2	99.2	99.2
ArB 13565	65.6	65.4	95.0	-	98.4	98.4	96.9	97.6	98.4	98.4	98.4
ArD 41519	65.1	65.1	92.4	93.2	-	98.4	96.9	96.9	98.4	98.4	98.4
P6-740	64.8	64.8	91.3	89.8	91.3	-	98.4	98.4	100.0	100.0	100.0
CPC-0740	63.5	63.5	90.8	89.5	89.5	96.1	-	96.9	98.4	98.4	98.4
EC YAP	63.3	63.3	90.3	89.8	89.5	95.3	97.4	-	98.4	98.4	98.4
H/PF/2013	64.0	63.5	91.6	90.3	89.8	95.5	98.4	97.1	-	100.0	100.0
Z1106033	63.8	63.3	91.9	90.6	90.0	95.8	98.2	96.9	99.7	-	100.0
PRVABC59	63.8	63.3	91.6	90.6	90.0	95.8	97.9	96.9	99.5	99.7	-

NS4b

	AR 94	*Chuku*	MR-766	ArB 13565	ArD 41519	P6-740	CPC-0740	EC YAP	H/PF/2013	Z1106033	PRVABC59
AR 94	-	100.0	80.0	81.2	81.6	82.0	81.2	80.8	81.2	81.2	81.2
Chuku	97.1	-	80.0	81.2	81.6	82.0	81.2	80.8	81.2	81.2	81.2
MR-766	69.5	69.7	-	98.0	96.8	97.2	95.2	94.4	94.8	94.8	94.8
ArB 13565	70.3	70.5	93.9	-	98.4	98.4	96.4	95.6	96.0	96.0	96.0
ArD 41519	71.4	71.6	92.6	93.2	-	98.0	96.8	96.0	96.4	96.4	96.4
P6-740	70.1	70.1	89.1	90.3	90.6	-	97.6	96.8	97.2	97.2	97.2
CPC-0740	71.4	70.8	87.4	87.8	89.0	95.0	-	98.8	99.2	99.2	99.2
EC YAP	71.1	70.8	87.8	88.2	89.1	95.0	98.3	-	98.8	98.8	98.8
H/PF/2013	70.8	70.8	87.6	87.8	89.0	94.7	97.9	98.3	-	100.0	100.0
Z1106033	70.8	70.8	87.5	87.6	89.1	94.7	97.7	98.1	99.7	-	100.0
PRVABC59	70.8	70.8	87.8	87.9	89.4	94.7	97.5	97.9	99.5	99.7	-